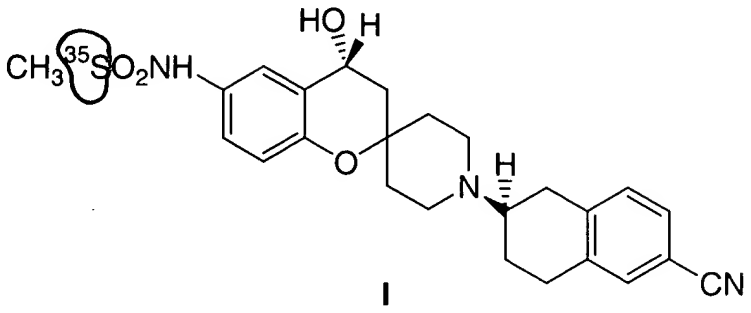
WHAT IS CLAIMED IS:

1. A radioligand compound of Formula I which is



I

Screen 2039
-abnormal mass - all isotopic specifications

or pharmaceutically acceptable salts thereof.

2. The radioligand compound of Formula I, as recited in Claim 1, which possesses a specific activity of greater than 500 Ci/mmol.

3. The radioligand compound of Formula I, as recited in Claim 1, which possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

4. A method of characterizing an ion channel as an I_{Kr} channel comprising contacting the ion channel with the radioligand compound of Claim 1 and determining if the radioligand compound binds to the ion channel.

5. A method for characterizing the activity of a compound as an I_{Kr} channel blocker comprising contacting the test compound with a membrane containing the I_{Kr} channel in the presence of the radioligand compound of Claim 1 and monitoring whether the test compound influences the binding of the radioligand compound to the membrane containing the I_{Kr} channel.

6. The method as recited in Claim 5, wherein the membrane containing the I_{Kr} channel is derived from a cell line transfected with the ERG gene.

7. The method as recited in Claim 6, wherein the cell line is HEK 293 cells or CHO cells.

8. The method as recited in Claim 7, wherein the ERG gene is human, canine or primate.

9. The method as recited in Claim 8, wherein the radioligand compound of Formula I possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

10. A method for assessing the binding of a test compound to a membrane containing the I_{Kr} channel using a radioligand compound of Formula I, [^{35}S]-radiolabeled (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydoxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide, comprising the steps of:

1) preparing solutions of the test compound at 5 or more different concentrations, a solution of control vehicle and a solution of (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydoxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide (compound of Formula II) in a solvent;

2) mixing the radioligand compound of Formula I with the membrane containing the I_{Kr} channel diluted with an assay buffer to form a membrane/radioligand mixture of known concentration;

3) incubating a quantity of known concentration of the membrane/radioligand mixture with the solution of test compound, control vehicle or compound of Formula II, as recited in Step 1, for a set time period at a temperature range of between about 4°C and about 37°C to give a mixture of membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II, where the final concentration of the membrane containing the I_{Kr} channel is predetermined;

4) isolating from the incubated mixture the membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II;

5) measuring the radioactivity of the isolated membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II;

6) repeating steps 3 through 5 with the test compound at each concentration, the solution of control vehicle and the solution of the compound of Formula II, as recited in Step 1; and

7) calculating the IC_{50} corresponding to the measured radioactivity of: 1) the membrane bound with the radioligand and each concentration of the test compound, 2) the membrane bound with the radioligand and with the control vehicle, and 3) the membrane bound with the radioligand and the compound of Formula II.

11. The method as recited in Claim 10, wherein the membrane containing the I_{Kr} channel is derived from a cell line transfected with the ERG gene.

12. The method as recited in Claim 11, wherein the cell line is HEK 293 cells or CHO cells.

13. The method as recited in Claim 12, wherein the ERG gene is human, canine or primate.

14. The method as recited in Claim 13, wherein the solutions of the test compound are prepared in Step 1 at 7 different concentrations.

15. The method as recited in Claim 14, wherein the time period for incubation in Step 3, is about 30 minutes to 1 hour.

16. The method as recited in Claim 15, wherein the temperature for the incubation in Step 3, is room temperature (25°C).

17. The method as recited in Claim 16, wherein the membrane-bound with radioligand or test compound is isolated in Step 4 with Unifilters, Scintillation Proximity Assay (SPA) beads or the Flashplates.

18. The method as recited in Claim 17, wherein the membrane containing the I_{Kr} channel is derived from a HEK 293 cell line transfected with the human ERG gene.

19. The method as recited in Claim 8, wherein the radioligand compound of Formula I possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

20. A method for assessing the binding of a test compound to a membrane containing the I_{Kr} channel using a radioligand of Formula I, [^{35}S]-radiolabeled (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydoxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide, comprising the steps of:

1) preparing assay wells with 4 μl of the test compound in dimethylsulfoxide (DMSO) diluted 100x with assay buffer at 5 or more different concentrations, a control vehicle of DMSO and a DMSO solution of (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydoxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide (compound of Formula II);

2) adding the radioligand compound of Formula I at 50pM to the membrane containing the I_{Kr} channel diluted with assay buffer to form a membrane/radioligand mixture;

3) incubating each assay well with 400 μl of the 50 pM membrane/radioligand mixture for about 75 minutes to about 90 minutes at room temperature (25^{o}C) to give assay wells containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II where the final concentration of the membrane containing the I_{Kr} channel is 11μg/ml;

4) filtering the incubated assay wells through 0.1% BSA presoaked filters to isolate on the filters the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II;

5) washing each of the filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II about 5 times with 500 μl of ice cold wash buffer;

6) drying the washed-filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II at room temperature in a fume hood;

7) adding 50 µl Microscint-20 microscintillate to the dried-filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II;

8) measuring the microscintillation count of the microscintillation-treated filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II for one minute; and

9) calculating the IC_{50} corresponding to the measured microscintillation count of: 1) the microscintillation-treated filters containing the membrane bound with the radioligand and each concentration of the test compound, 2) the microscintillation-treated filters containing the membrane bound with the radioligand and with the control vehicle, and 3) the microscintillation-treated filters containing the membrane bound with the radioligand and the compound of Formula II.

21. The method as recited in Claim 20, wherein the membrane containing the I_{Kr} channel is derived from a cell line transfected with the ERG gene.

22. The method as recited in Claim 21, wherein the cell line is HEK 293 cells.

23. The method as recited in Claim 22, wherein the ERG gene is human or canine.

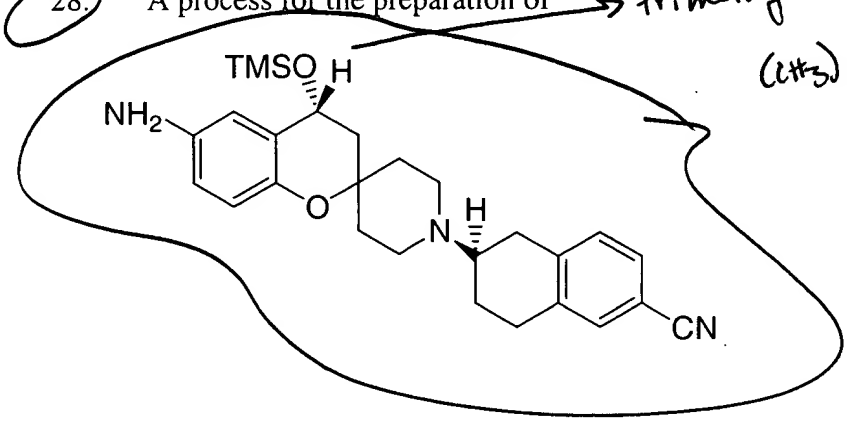
24. The method as recited in Claim 23, wherein the solutions of the test compound are prepared in Step 1 at 7 different concentrations.

25. The method as recited in Claim 24, wherein the membrane containing the I_{Kr} channel is derived from a HEK 293 cell line transfected with the human ERG gene.

26. The method as recited in Claim 25, wherein the radioligand compound of Formula I possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

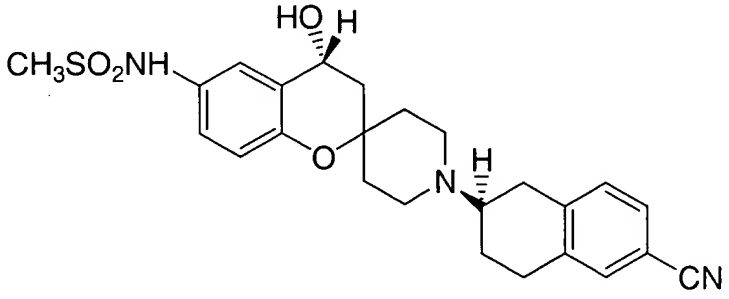
27. The method as recited in Claim 26, wherein the membrane-bound with radioligand or test compound is filtered in Step 4 with Unifilters.

28. A process for the preparation of → trimethylsilyloxy $(CH_3)_3SiO-$

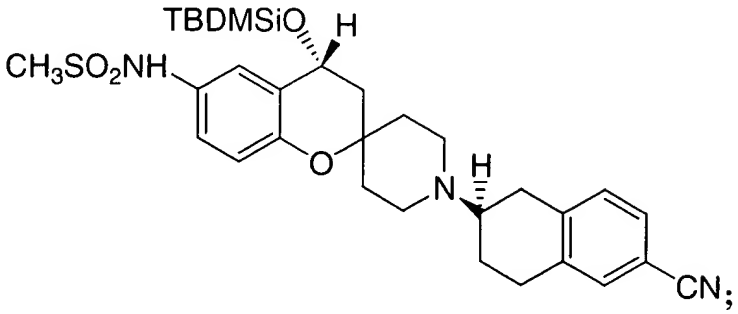


comprising the steps of

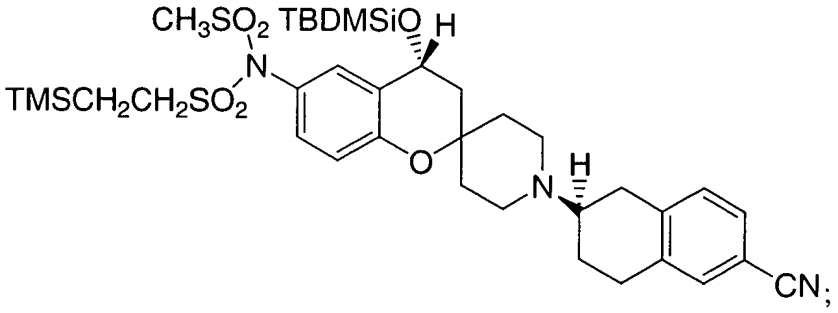
(1) reacting the alcohol with 2,6-lutidine and t-butyldimethylsilyl triflate



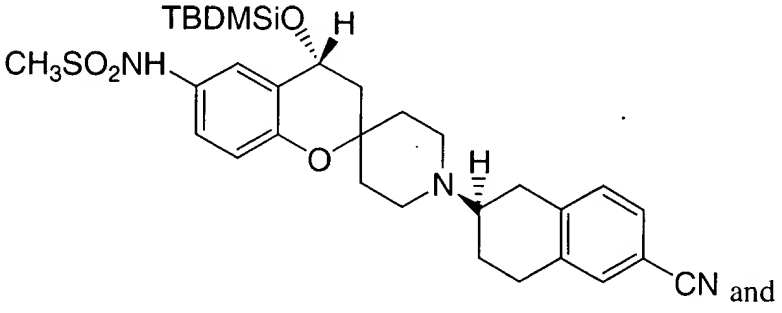
to give a t-butyldimethylsilyl-protected hydroxyl compound

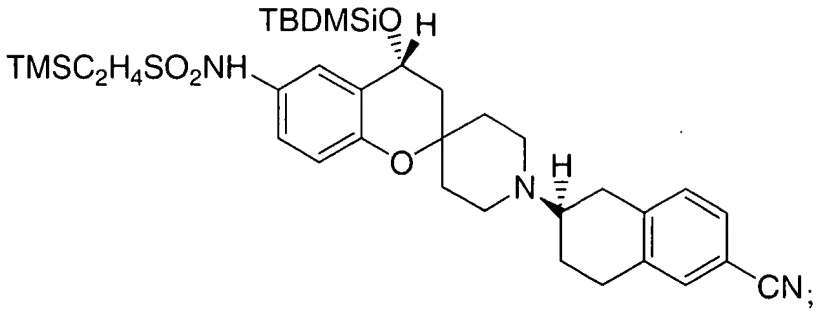


(2) alkylating the t-butyldimethylsilyl-protected hydroxyl compound by treating with sodium hydride, and then treating with 2-trimethylsilylethanesulfonylchloride to give the disubstituted sulfonamide compound

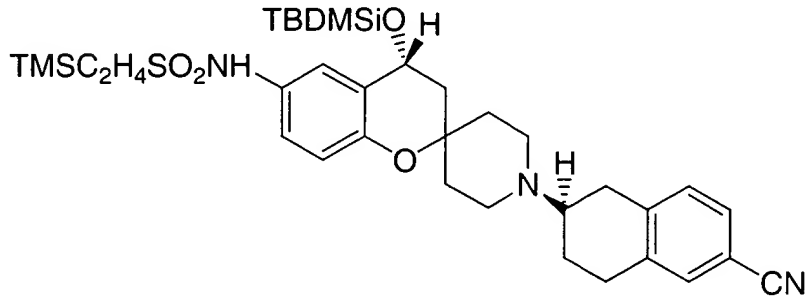


(3) treating the disulfonamide with an C_1-C_8-alkanethiolate to give a mixture of the following sulfonamides;

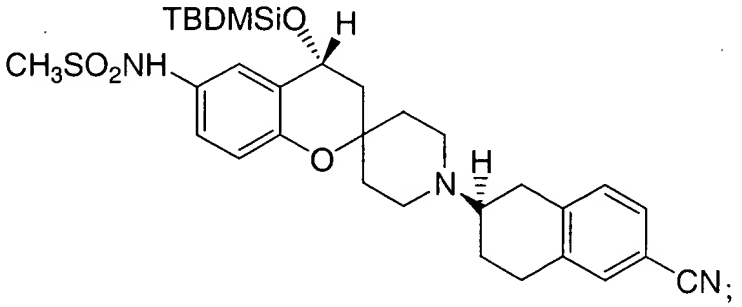




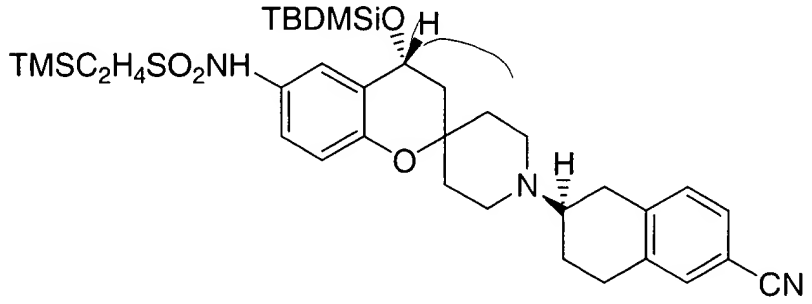
(4) separating the sulfonamide mixture using chromatography to isolate the non-polar sulfonamide eluting with a non-polar solvent system



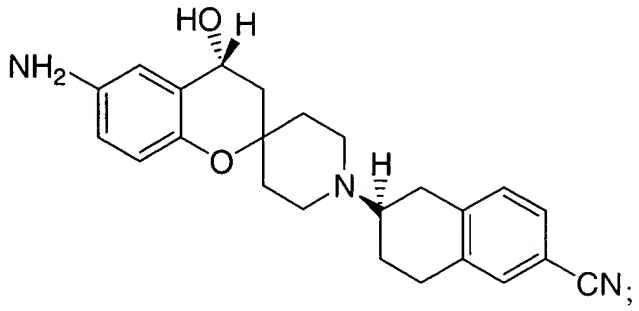
and then eluting off the polar isomer using a polar solvent system



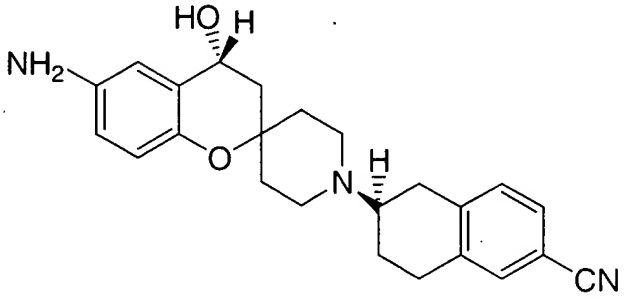
(5) desulfonating the non-polar isomer



using a fluoride compound in an organic base and heating for about 24 hours to about 48 hours to give the free alcohol-amine



(6) reacting the free alcohol-amine with trimethylsilyl imidazole in an organic solvent



to give the desired trimethylsilyloxy compound.

29. The process as recited in Claim 28, wherein the alkylation in step 2 is stirred at room temperature for up to 24 hours.

30. The process as recited in Claim 29, wherein the C_1-C_8-alkanethiolate in step 3 is sodium methanethiolate, sodium ethanethiolate, sodium propanethiolate or sodium 2-methylpropanethiolate.

31. The process as recited in Claim 30, wherein the desulfonylation reaction in step 3 is run for less than 24 hours.

32. The process as recited in Claim 31, wherein the separation of the sulfonamide mixture in step 4 is run using flash chromatography with a solvent system of ethyl acetate and hexane to ethyl acetate and methanol.

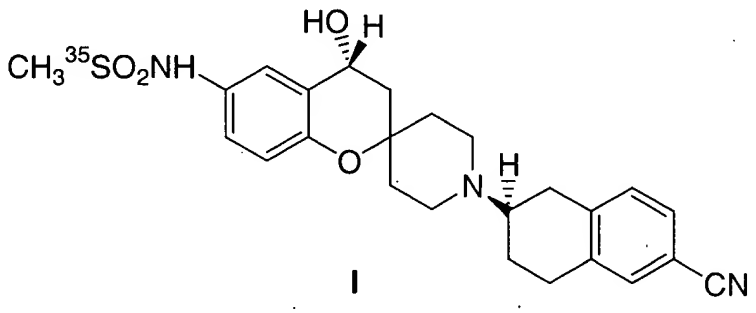
33. The process as recited in Claim 32, wherein the separation of the sulfonamide mixture in step 4 is run using flash chromatography with a non-polar solvent system of 1:1 ethyl acetate: hexane to a polar solvent system of 99:1 ethyl acetate: methanol.

34. The process as recited in Claim 33, wherein The fluoride compound used in the desulfonylation of step 5 is selected from: cesium fluoride and tetrabutylammonium fluoride.

35. The process as recited in Claim 34, wherein the organic solvent used in the desulfonylation of step 5 is selected from: dimethylformamide, dimethylsulfoxide and N-methylpyrrolidinone.

36. The process as recited in Claim 35, wherein the organic solvent in step 6 is acetonitrile, tetrahydrofuran, or ether.

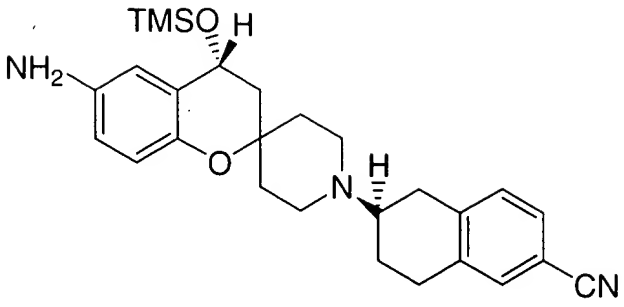
37. A process for the preparation of a radioligand compound of Formula I



I

comprising the steps of:

(a) reacting the amine



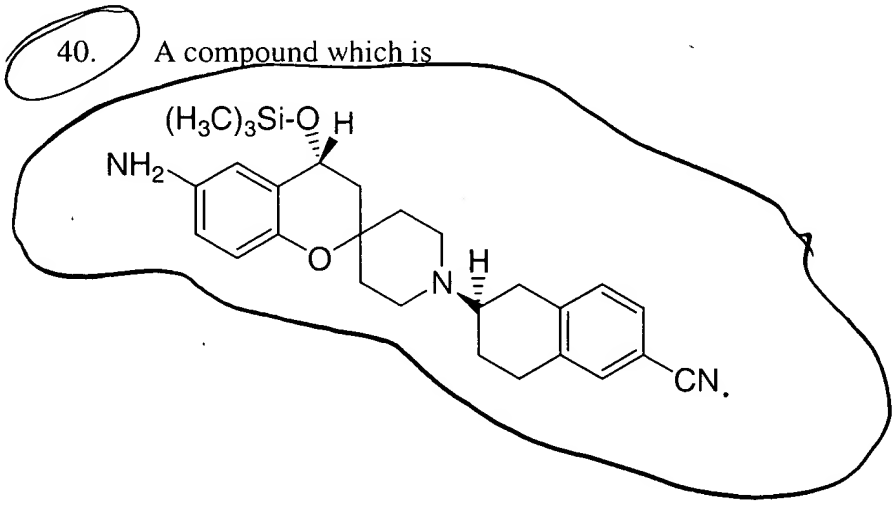
with [^{35}S]-methanesulfonyl chloride in the presence of an organic base to form the silyl-protected [^{35}S]-methanesulfonamide; and

(b) removing the silyl-protecting group of the silyl-protected [^{35}S]-methanesulfonamide with trifluoroacetic acid to give the radioligand compound of Formula I.

38. The process as recited in Claim 37, wherein the organic base is selected from triethylamine, trimethylamine, and diisopropylethylamine.

39. The process as recited in Claim 38, wherein the organic base is triethylamine.

40. A compound which is



Claim 28 Structure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L12                 STR
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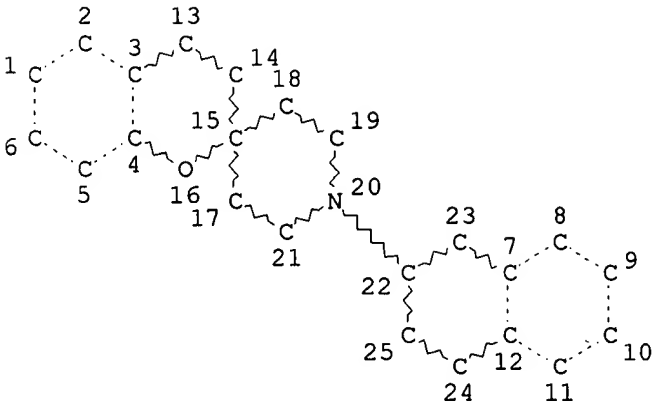

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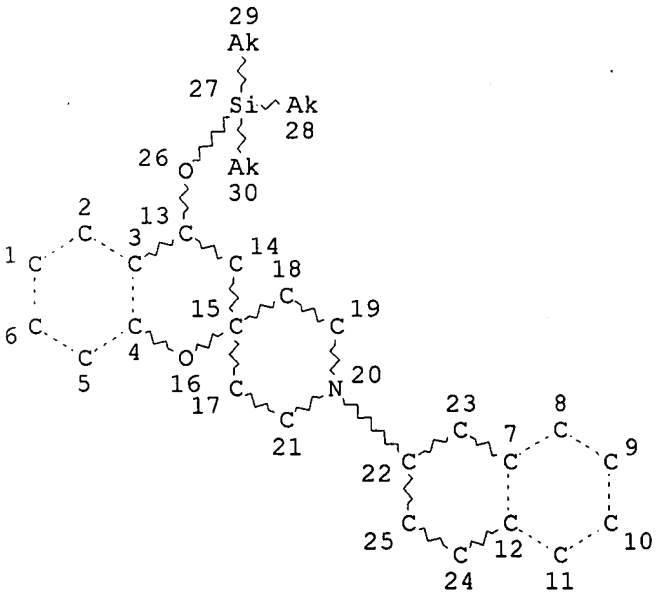

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L28 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:71916 HCAPLUS
DOCUMENT NUMBER: 136:112612
TITLE: Radioligand and binding assay
INVENTOR(S): Butcher, John W.; Claremon, David A.; Connolly, Thomas M.; Dean, Dennis C.; Karczewski, Jerzy; Koblan, Kenneth S.; Kostura, Matthew J.; Liverton, Nigel J.; Melillo, David G.
PATENT ASSIGNEE(S): Merck & Co., Inc., USA
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002005860	A1	20020124	WO 2001-US21731	20010710
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US 2002034730	A1	20020321	US 2001-904045	20010712

PRIORITY APPLN. INFO.: US 2000-218397P P 20000714

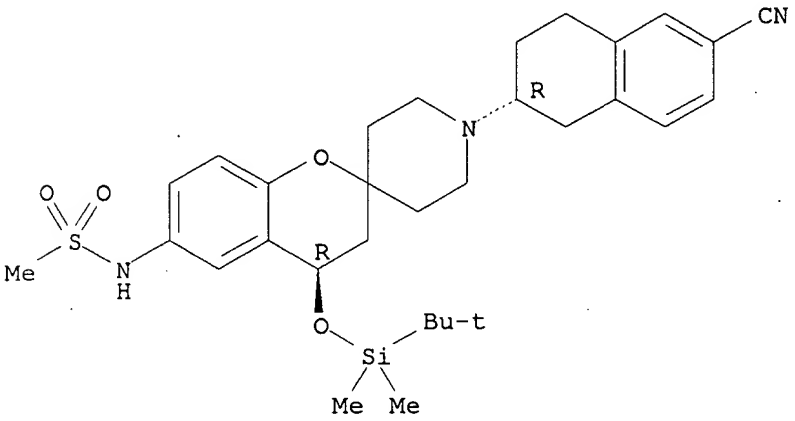
AB The present invention is directed to the radioligand compd., [35S]-radiolabeled (+)-N-[1 -(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxyspiro[2H- -benzopyran-2,4 -piperidin]-6-yl]methanesulfonamide, its prepn., its use in characterizing an ion channel as an IKr channel, and its potential use in screening for Class III antiarrhythmic activity.

IT **390388-46-2P 390388-47-3P 390388-49-5P 390388-51-9P**
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(prepn. of 35S-labeled IKr channel ligand as potential screening agent for antiarrhythmics)

RN 390388-46-2 HCAPLUS
CN Methanesulfonamide, N-[(4R)-1'-[(2R)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-

dihydrospiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



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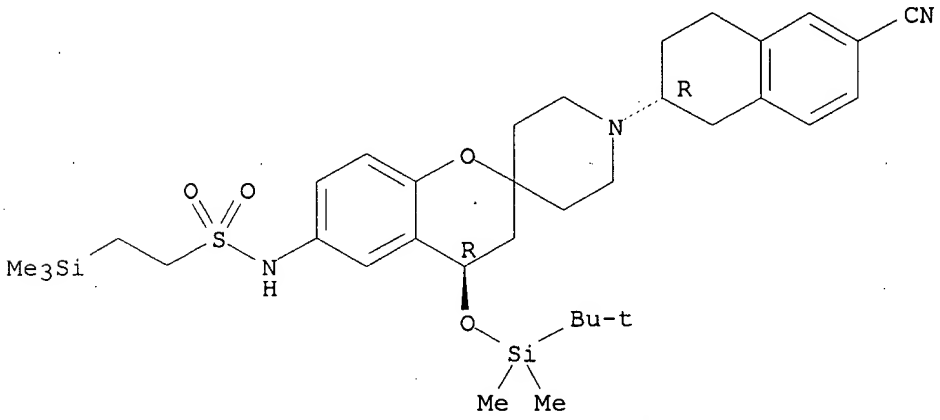
Absolute stereochemistry.



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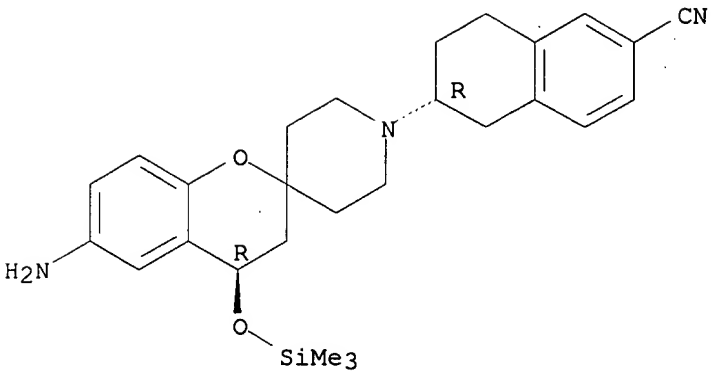
Absolute stereochemistry.



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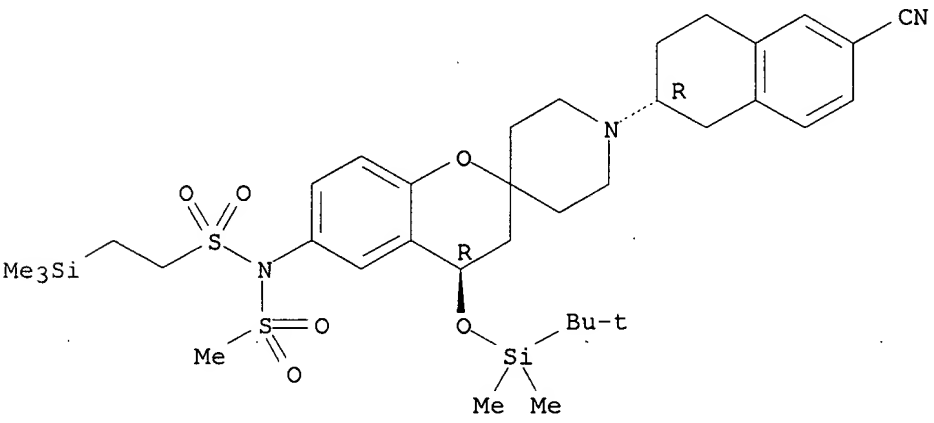
Absolute stereochemistry.



REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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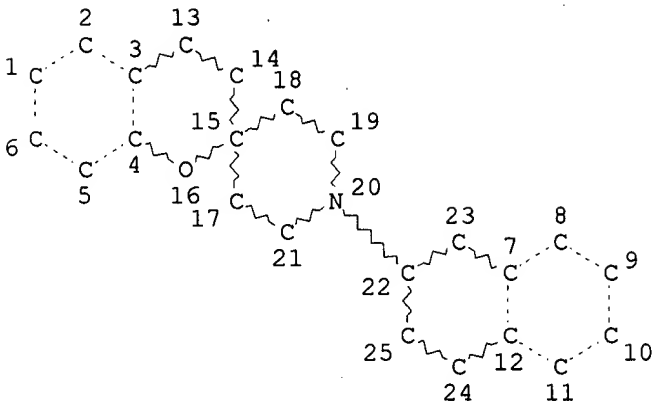

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NUMBER OF NODES IS 25

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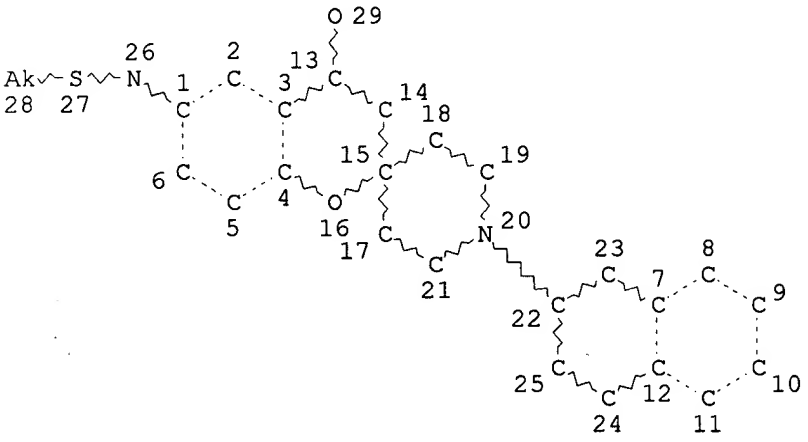

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L24 ANSWER 1 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:71916 HCAPLUS
DOCUMENT NUMBER: 136:112612
TITLE: Radioligand and binding assay
INVENTOR(S): Butcher, John W.; Claremon, David A.; Connolly, Thomas M.; Dean, Dennis C.; Karczewski, Jerzy; Koblan, Kenneth S.; Kostura, Matthew J.; Liverton, Nigel J.; Melillo, David G.
PATENT ASSIGNEE(S): Merck & Co., Inc., USA
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002005860	A1	20020124	WO 2001-US21731	20010710
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002034730	A1	20020321	US 2001-904045	20010712

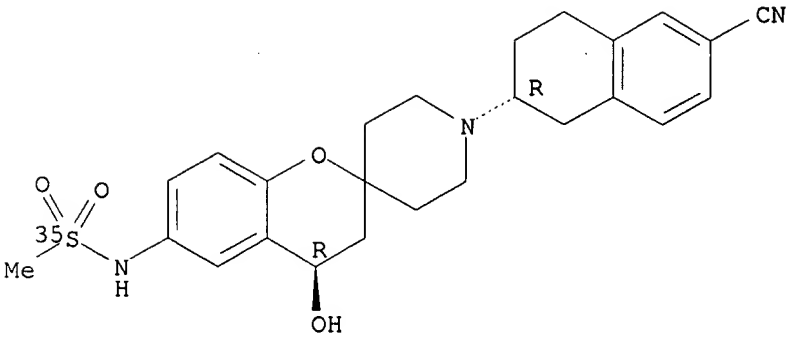
PRIORITY APPLN. INFO.: US 2000-218397P P 20000714

AB The present invention is directed to the radioligand compd., [35S]-radiolabeled (+)-N-[1 -(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxyspiro[2H- -benzopyran-2,4 -piperidin]-6-yl]methanesulfonamide, its prepn., its use in characterizing an ion channel as an **IKr** channel, and its potential use in screening for Class III antiarrhythmic activity.

IT **390388-50-8P**
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(prepn. of 35S-labeled **IKr** channel ligand as potential screening agent for antiarrhythmics)

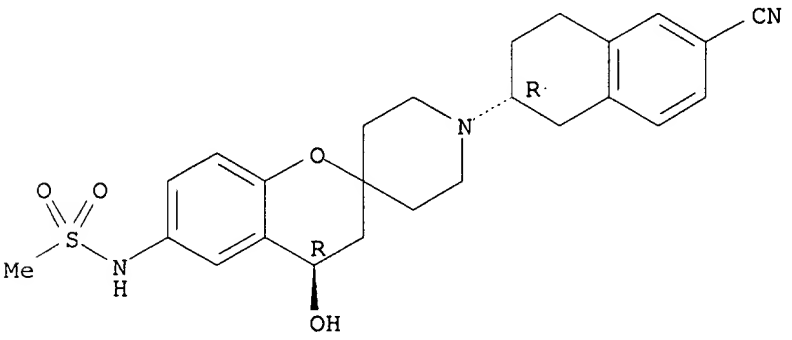
RN 390388-50-8 HCAPLUS
CN Methanesulfonamide-35S, N-[(4R)-1'-[(2R)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



IT **161799-18-4**
RL: RCT (Reactant); RACT (Reactant or reagent)
(prepn. of 35S-labeled **IKr** channel ligand as potential screening agent for antiarrhythmics)
RN 161799-18-4 HCAPLUS
CN Methanesulfonamide, N-[1'-(6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl)-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, [R-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L24 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:220902 HCAPLUS
DOCUMENT NUMBER: 126:304196
TITLE: Single HERG delayed rectifier K+ channels expressed in Xenopus oocytes
AUTHOR(S): Zou, Anruo; Curran, Mark E.; Keating, Mark T.; Sanguinetti, Michael C.
CORPORATE SOURCE: Cardiology Division, Univ. Utah Health Sciences Center, Salt Lake City, UT, 84132, USA
SOURCE: American Journal of Physiology (1997), 272(3, Pt. 2), H1309-H1314
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal

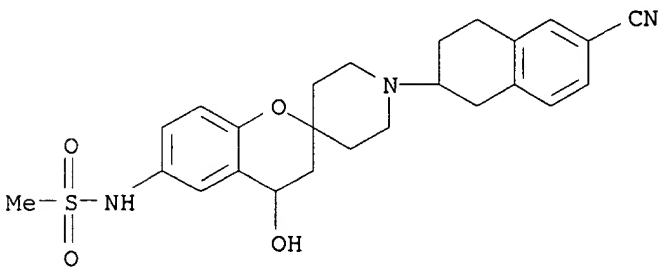
LANGUAGE: English

AB HERG is a K+ channel with properties similar to the rapidly activating component (**IKr**) of delayed rectifier K+ current, which is important for repolarization of human cardiac myocytes. In this study, the authors have characterized the single-channel properties of HERG expressed in Xenopus oocytes. Currents were measured in cell-attached patches with an extracellular K concn. of 120 mM. The single HERG channel conductance, detd. at test potentials between -50 and -110 mV, was 12.1 pS. At pos. test potentials (40 to 80 mV), the probability of channel opening was low and slope conductance was 5.1 pS. The mean channel open times at -90 mV were 2.9 and 11.8 ms, and the mean channel closed times were 0.54 and 14.5 ms. Single HERG channels were blocked by MK-499, a class III antiarrhythmic agent that blocks **IKr** in cardiac myocytes. The development of block was more rapid in inside-out patches than in cell-attached patches or in whole cell recordings, indicating that block occurs from the cytoplasmic side of the membrane. The single-channel properties of HERG are similar to **IKr** channels of isolated cardiac myocytes, which provides further evidence that HERG proteins coassemble to form **IKr** channels.

IT **150481-98-4**, MK-499
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(single HERG delayed rectifier K+ channels expressed in Xenopus oocytes)

RN 150481-98-4 HCAPLUS

CN Methanesulfonamide, N-[(4R)-1'-[(2R)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, rel- (9CI) (CA INDEX NAME)



L24 ANSWER 3 OF 6 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1996:425284 HCAPLUS
DOCUMENT NUMBER: 125:86621
TITLE: Preparation of N-[1'-(6-cyano-4-hydroxy-1,2,3,4-tetrahydro-2(R)-naphthalenyl-3,4-dihydro-4(R)-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin-6-yl]methanesulfonamide as an antiarrhythmic agent.
INVENTOR(S): Slaughter, Donald E.
PATENT ASSIGNEE(S): Slaughter, Donald, E., USA
SOURCE: PCT Int. Appl., 22 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9607411	A1	19960314	WO 1995-US11348	19950905
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, US, UZ				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9535852	A1	19960327	AU 1995-35852	19950905
PRIORITY APPLN. INFO.:			US 1994-301946	19940907
			WO 1995-US11348	19950905

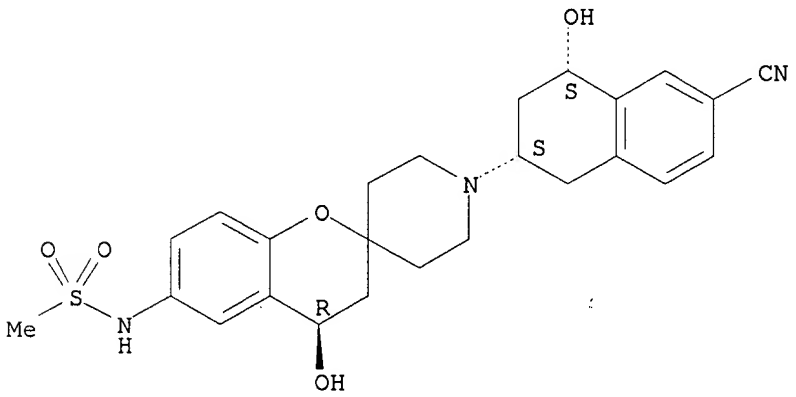
AB Title compd. (I; R = OH)(II) was prepd. Thus, I (R = H) was incubated with non-induced rat liver microsomes in the presence of glucose-6-phosphate, NADP, glucose-6-phosphate dehydrogenase, potassium phosphate buffer, and MgCl2 at 37.degree. to give II, which had an IC50 <1000 nM as an **IKr** blocker.

IT **178470-87-6P 178737-88-7P**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(prepn. of N-[1'-(6-cyano-4-hydroxy-1,2,3,4-tetrahydro-2(S)-naphthalenyl-3,4-dihydro-4(R)-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin-6-yl]methanesulfonamide as an antiarrhythmic agent)

RN 178470-87-6 HCAPLUS

CN Methanesulfonamide, N-[1'-(6-cyano-1,2,3,4-tetrahydro-4-hydroxy-2-naphthalenyl)-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, [2S-[2.alpha.(S*),4.alpha.]]- (9CI) (CA INDEX NAME)

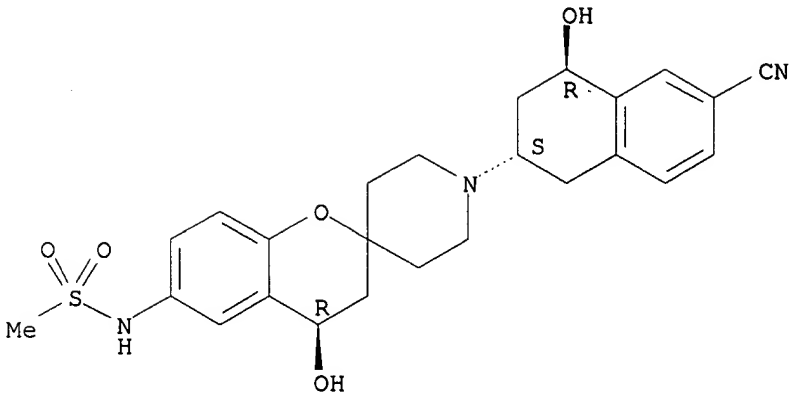
Absolute stereochemistry. Rotation (+).



RN 178737-88-7 HCAPLUS

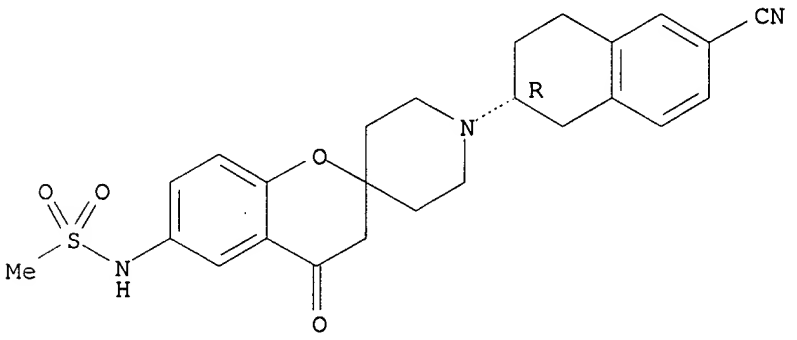
CN Methanesulfonamide, N-[1'-(6-cyano-1,2,3,4-tetrahydro-4-hydroxy-2-naphthalenyl)-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, [2S-[2.alpha.(S*),4.beta.]]- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



IT **136081-07-7P**
RL: SPN (Synthetic preparation); PREP (Preparation)
(prepn. of N-[1'-(6-cyano-4-hydroxy-1,2,3,4-tetrahydro-2(S)-naphthalenyl-3,4-dihydro-4(R)-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin-6-yl]methanesulfonamide as an antiarrhythmic agent)
RN 136081-07-7 HCAPLUS
CN Methanesulfonamide, N-[1'-(6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl)-3,4-dihydro-4-oxospiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, (R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



L24 ANSWER 4 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:293219 HCAPLUS
DOCUMENT NUMBER: 125:901
TITLE: Differential efficacy of the class III agent MK-499 against programmed stimulation-induced and ischemic-induced ventricular arrhythmias in a canine model of previous myocardial infarction
AUTHOR(S): Lynch, Joseph J., Jr.; Wallace, Audrey A.; Stump, Gary L.; Stupienski, Raymond F., III; Kothstein, Theresa; Gehret, John R.
CORPORATE SOURCE: Dep. Pharmacology and Lab. Animal Resources, Merck Res. Lab., West Point, PA, USA
SOURCE: Journal of Pharmacology and Experimental Therapeutics (1996), 277(2), 671-678

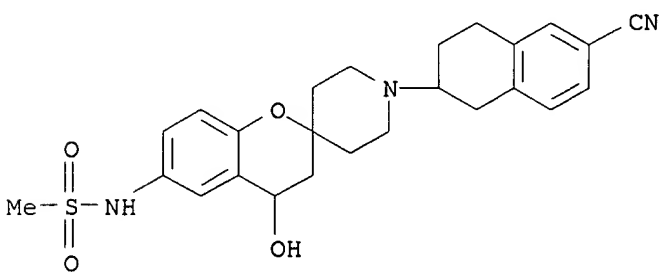
CODEN: JPETAB; ISSN: 0022-3565
PUBLISHER: Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Class III activity has been proposed as potential mechanism for the treatment of reentrant arrhythmias. The purpose of the present study was to assess the concordance in antiarrhythmic efficacy of MK-499, a selective blocker of **IKr**, the rapidly activating component of cardiac delayed rectifier K+ current, against programmed ventricular stimulation (PVS)-induced ventricular tachycardias and thrombotically induced lethal ischemic arrhythmias, and to characterize the electrophysiol. determinants of antiarrhythmic efficacy in a canine model of previous myocardial infarction. Single i.v. doses of 1.0, 3.0 and 10.0 .mu.g/kg MK-499 were administered to anesthetized dogs with anterior myocardial infarctions. Protection (suppression + stabilizing/slowing) vs. PVS-induced ventricular tachycardias occurred in 5/11 (45%) prepns. at 1.0 .mu.g/kg, in 9/12 (75%) prepns. at 3.0 .mu.g/kg and in 10/11 (91%) prepns. at 10.0 .mu.g/kg i.v. MK-499. The incidences of lethal ventricular arrhythmias developing in response to thrombotically induced posterolateral myocardial ischemia were 34/40 (85%) in vehicle controls, 7/11 (64%) at 1.0 .mu.g/kg, 6/12 (50%,) at 3.0 .mu.g/kg and 4/11 (36%,) at 10.0 .mu.g/kg i.v. MK-499. Low-dose i.v. MK-499 prolonged ECG QT interval and increased noninfarct zone and infarct zone ventricular refractoriness. However, there was a poor concordance (56%) between response to PVS with MK-499 and response to thrombotically induced acute myocardial ischemia. Furthermore, different trends of assocn. between site and magnitude of Class III effect and antiarrhythmic efficacy were obsd. for PVS- vs. ischemia-induced arrhythmias. Hence, although low-dose i.v. MK-499 provided significant protection against both elec. and ischemia-triggered arrhythmias in the setting of previous myocardial infarction, protection against PVS-induced ventricular tachycardias was not highly predictive of protection against lethal ischemic arrhythmias in this prepn.

IT **150481-98-4**, MK-499
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(differential efficacy of class III agent MK-499 against programmed stimulation-induced and ischemic-induced ventricular arrhythmias in a canine model of previous myocardial infarction)

RN 150481-98-4 HCAPLUS

CN Methanesulfonamide, N-[(4R)-1'-[(2R)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, rel- (9CI) (CA INDEX NAME)

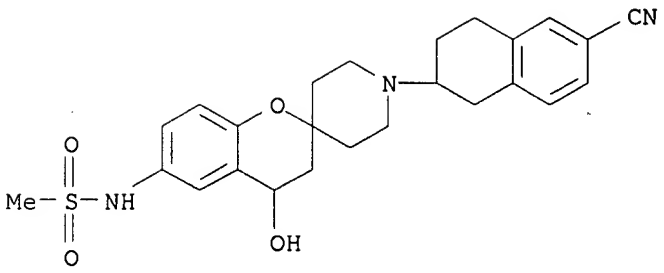


L24 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:131858 HCAPLUS
DOCUMENT NUMBER: 124:220034
TITLE: Class III antiarrhythmic drugs block HERG, a human cardiac delayed rectifier K+ channel: open-channel block by methanesulfonanilides
AUTHOR(S): Spector, Peter S.; Curran, Mark E.; Keating, Mark T.; Sanguinetti, Michael C.
CORPORATE SOURCE: Dep. Human Genetics, Univ. Utah Health Sci. Cent., Salt Lake City, UT, USA
SOURCE: Circulation Research (1996), 78(3), 499-503
CODEN: CIRUAL; ISSN: 0009-7330
PUBLISHER: American Heart Association
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors recently reported that mutations in HERG, a potassium channel gene, cause long QT syndrome. Heterologous expression of HERG in Xenopus oocytes revealed that this channel had biophys. properties nearly identical to a cardiac delayed rectifier K+ current, **IKr**, but had dissimilar pharmacol. properties. Class III antiarrhythmic drugs such as E-4031 and MK-499 are potent and specific blockers of **IKr** in cardiac myocytes. The authors initial studies indicated that these compds. did not block HERG at a concn. of 1 .mu.mol/L. In the present study, the authors used std. two-microelectrode voltage-clamp techniques to further characterize the effects of these drugs on HERG channels expressed in oocytes. Consistent with initial findings, 1 .mu.mol/L MK-499 and E-4031 had no effect on HERG when oocytes were voltage clamped at a neg. potential and not pulsed during equilibration with the drug. However, MK-499 did block HERG current if oocytes were repetitively pulsed, or clamped at a voltage pos. to the threshold potential for channel activation. This finding is in contrast to previous studies that showed significant block of **IKr** in isolated myocytes by similar drugs, even in the absence of pulsing. This apparent discrepancy may be due to differences in channel characteristics (HERG vs. guinea pig and mouse **IKr**), tissue (oocytes vs. myocytes), or specific drugs. Under steady state conditions, block of HERG by MK-499 was half maximal at 123 nmol/L at a test potential of -20 mV. MK-499 (150 nmol/L) did not affect the voltage dependence of activation and rectification nor the kinetics of activation and rectification nor the kinetics of activation and deactivation of HERG. These data indicate that MK-499 preferentially blocks open HERG channels and further support the conclusion that HERG subunits form **Ikr** channels in cardiac myocytes.

IT **150481-98-4**, MK-499
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(class III antiarrhythmic methanesulfonanilides block human cardiac delayed rectifier K+ channel HERG by open-channel block)

RN 150481-98-4 HCAPLUS
CN Methanesulfonamide, N-[(4R)-1'-[(2R)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, rel- (9CI) (CA INDEX NAME)



L24 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1994:548695 HCAPLUS
DOCUMENT NUMBER: 121:148695
TITLE: Comparative effects of increased extracellular potassium and pacing frequency on the class III activities of methanesulfonanilide **IKr** blockers dofetilide, D-Sotalol, E-4031, and MK-499
AUTHOR(S): Baskin, Elizabeth P.; Lynch, Joseph J., Jr.
CORPORATE SOURCE: Dep. Pharmacol., Merck Res. Lab., West Point, PA, USA
SOURCE: Journal of Cardiovascular Pharmacology (1994), 24(2), 199-208
CODEN: JCPCDT; ISSN: 0160-2446
DOCUMENT TYPE: Journal
LANGUAGE: English

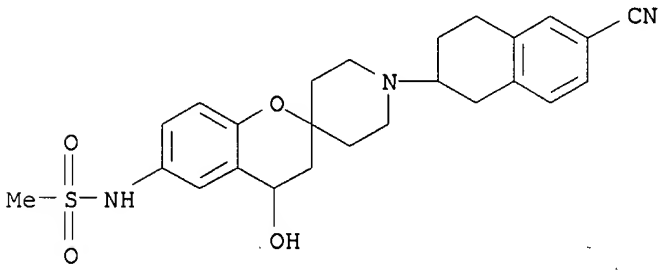
AB The methanesulfonanilide-contg. Class III agents dofetilide, D-sotalol, E-4031, and MK-499 have been characterized as selective blockers of a rapidly activating component of the cardiac delayed rectifier (IK) K+ current, **IKr**. In the present studies, the effects of dofetilide (3-30 nM), D-sotalol (10-100 .mu.M), E-4031 (30-300 nM), and MK-499 (30-300 nM) on myocardial effective refractory period (ERP) were assessed in ferret right ventricular papillary muscles in conditions of altered extracellular K+ concn. [K+]e [normal (4 mM) vs. increased (10 mM)] concns., and of altered pacing frequency (1-3 Hz). With 4 mM [K+]e, all four agents elicited significant, concn.-dependent ERP increases in the frequency range of 1-3 Hz, and all four agents displayed reverse frequency-dependent activity. Reverse frequency-dependent profiles also were demonstrable in 10 mM [K+]e at the higher test agent concns.: dofetilide (10 and 30 nM), D-sotalol (100 .mu.M), E-4031 (100 and 300 nM) and MK-499 (100 and 300 nM). All four agents displayed diminished ERP increases in increased vs. normal [K+]e. Among individual test agents, however, there were differences in magnitudes of diminution of ERP increases obsd. in increased [K+]e: the activities of D-sotalol and MK-499 were better maintained in increased [K+]e than were those of dofetilide and E-4031. As a result of this differential sensitivity increased [K+]e, significant ERP increases were not demonstrable for dofetilide and E-4031 in simultaneous conditions of increased [K+]e and rapid pacing, whereas significant activities were maintained with D-sotalol and MK-499 in increased [K+]e throughout the 1-3 Hz range of pacing frequencies. However, the inherent tendency of myocardial refractoriness to increase in increased [K+]e, particularly at faster pacing frequencies, played a dominant role in detg. the relation between increased vs. normal [K+]e post-treatment ERP in all Class III treatment groups. This frequency-dependent increment in refractoriness in increased [K+]e reflected in baseline ERP detd. in 10 vs. 4 mM [K+]e, resp., at frequencies of 1 Hz (163 vs. 157 ms), 2 Hz (146 vs. 134 ms), and 3 Hz (134

vs. 112 ms) tended to offset as well as minimize differences among the **IKr** blockers in diminution of activity obsd. in increased [K+]e. As a consequence, no fundamental differences among the methanesulfonanilide IKf blockers were apparent with regard to the influence of altered pacing frequency and [K+]e on effects on abs. refractoriness in this exptl. prepn.

IT **150481-98-4**, MK 499
RL: BIOL (Biological study)
(potassium response to, comparison with other class III antiarrhythmics)

RN 150481-98-4 HCAPLUS

CN Methanesulfonamide, N-[(4R)-1'-[(2R)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-, rel- (9CI) (CA INDEX NAME)



Radioligand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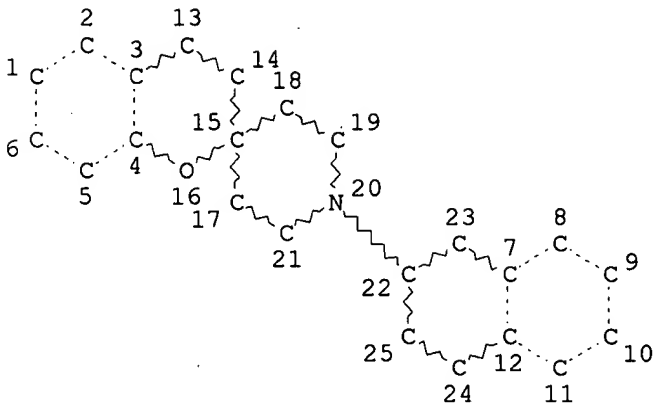

Broad Parent Structure
- all ring nodes open to all substitution

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NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS  25

STEREO ATTRIBUTES: NONE
L13          238 SEA FILE=REGISTRY ABB=ON  PLU=ON  NC5-OC5-C6/ES AND C6-C6/ES
L14          178 SEA FILE=REGISTRY SUB=L13 SSS FUL L12
L15              SCR 2039
L16            1 SEA FILE=REGISTRY SUB=L14 SSS FUL L12 AND L15
L17            1 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L16
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— Screen 2039 → abnormal mass - all isotopes

← only one isotopically labelled structure.

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=> d ibib ab hitstr

L17  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2002:71916  HCAPLUS
DOCUMENT NUMBER:          136:112612
TITLE:                    Radioligand and binding assay
INVENTOR(S):              Butcher, John W.; Claremon, David A.; Connolly, Thomas
                          M.; Dean, Dennis C.; Karczewski, Jerzy; Koblan,
                          Kenneth S.; Kostura, Matthew J.; Liverton, Nigel J.;
                          Melillo, David G.
PATENT ASSIGNEE(S):       Merck & Co., Inc., USA
SOURCE:                   PCT Int. Appl., 35 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
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     WO 2002005860      A1   20020124      WO 2001-US21731  20010710
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
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CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2002034730 A1 20020321 US 2001-904045 20010712

PRIORITY APPLN. INFO.: US 2000-218397P P 20000714

AB The present invention is directed to the radioligand compd., [35S]-radiolabeled (+)-N-[1 -(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxyspiro[2H- -benzopyran-2,4 -piperidin]-6-yl]methanesulfonamide, its prepn., its use in characterizing an ion channel as an IKr channel, and its potential use in screening for Class III antiarrhythmic activity.

IT **390388-50-8P**

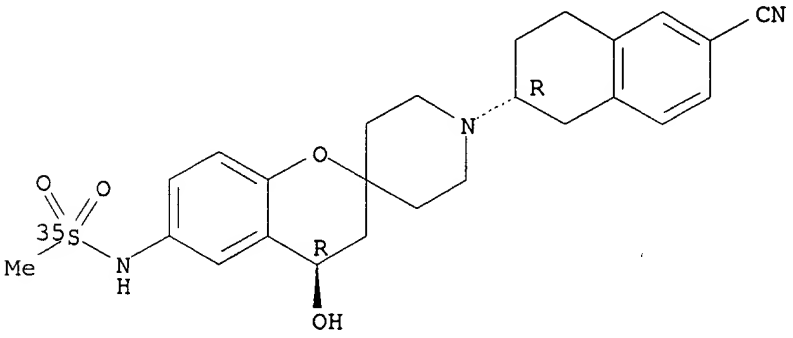
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)

(prepn. of 35S-labeled IKr channel ligand as potential screening agent for antiarrhythmics)

RN 390388-50-8 HCAPLUS

CN Methanesulfonamide-35S, N-[(4R)-1'-[(2R)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



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